Original Article

Exposure to Global System for Mobile Communication 900 MHz Cellular Phone Radiofrequency Alters Growth, Proliferation and Morphology of Michigan Cancer Foundation-7 Cells and Mesenchymal Stem Cells

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Abstract

Background: Today, using cellular phone and its harmful effects in human life is growing. The aim of this study is to investigate the effect of the global system for mobile communication (GSM) 900 MHz cellular phone radiofrequency waves on growth, morphology, and proliferation rate of mesenchymal stem cells and Michigan Cancer Foundation (MCF-7) cells within the specific distance and intensity. **Methods:** MCF-7 and human adipose-derived stem cells (HADSCs) were exposed to GSM cellular phones 900 MHz frequency with intensity of 354.6 μW/cm^2 during different exposure times 6, 21, 51, and 101 min/day with an interval of 10 min for each subsequent radiation exposure for 3 and 5 days at 10 and 20 cm distances from antenna. 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide assay and trypan blue test were used to determine the growth of cells and cell viability, respectively. Statistical analyses were carried out using three-way ANOVA. Differences were significant when $P < 0.05$. **Results:** The proliferation rates of both MCF-7 and HADSCs cells in all exposure groups were significantly lower than controls ($P < 0.05$). There was a significant effect on the percentage of cell survival with increase the period of time from 3 to 5 days for MCF-7 ($P < 0.01$) and HADSCs ($P = 0.02$), respectively. Variations in distance had no significant effect on the percentage of cell survival ($P = 0.35$) on MCF-7 ($P = 0.02$) and HADSCs ($P = 0.09$) cells, respectively. **Conclusions:** The results showed that radiation of GSM 900 MHz cellular phone may be reduced cell viability and proliferation rates of both cells. It is recommended to reduce exposure time, increase distance from antenna, and reserve the use of cell phones for shorter conversations to prevent its biological and harmful effects. Further studies with other intensities and frequencies on different cells are recommended.

Keywords: *Cell phones, cell proliferation, electromagnetic fields, neoplasms, stem cells*

Introduction

The effects of nonionizing radiofrequency electromagnetic field (RF-EMF) exposure on biological systems, due to its potential health hazards, have become the focus of interest since many years ago.[1,2] In recent years, with the development of microwave (frequencies ranging from 300 MHz to 300 GHz) devices including mobile phones, its harmful effects are causing great concern with human.[3-6]

Specific absorption rate (SAR) is the quantity of the rate at which energy is absorbed by the human body when exposed to an RF-EMF indicated the amount of heat generated in each kilogram of body tissues.[7] Global system for mobile communication (GSM) 900 mobile phone system has 900–1800 MHz frequency range with power 1–2 W and 217 Hz modulation frequency to transmit data at a bandwidth of 200 kHz.[8] The cellular and molecular changes due to RF-EMF radiation depend on some factors such as duration of radiation, the amount of permeability in tissues, and generation of heat. In addition, these factors depend on the intensity and frequency of waves.[9] Several studies concentrated on selecting radiation dose with changes in the frequency, radiation intensity, irradiation protocol, and the distance from source of RF-EMF.[6-15]

Mesenchymal (human adipose-derived) stem cells are one of the most important stem cell lines in tissue engineering and cell treatment because of the capacity of self-renewal and differentiation into

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specialized cells. These cells are found in the bone marrow, cord blood umbilical, and adipose tissue.[11,12] Michigan Cancer Foundation (MCF)-7 cell has high proliferation capacity and is very resistant, and features preserve the breast epithelium.[13-15]

Although studies on the effects of GSM 900 MHz exposure on proliferation rate of MCF-7 cells in shorter exposure time (<30 min) exist,[16] there is no evidence due to the influence of GSM 900 MHz exposure on growth and proliferation rate of human adipose-derived stem cells (HADSCs) and MCF-7 cells and comparison their results. To the best of our knowledge, this work is the first to investigate the effects of GSM 900 MHz on growth and proliferation rate of both HADSCs and MCF-7 cells with intensity of 354.6 μW/cm^2, during different exposure times. The aim of this study is to investigate the effects of the GSM 900 MHz cellular phone radiofrequency waves on growth, morphology, and proliferation rate of mesenchymal stem cells and MCF-7 cells within different distances and duration times.

Methods

Exposure system

RF-EMF exposure system was designed and fabricated by researcher team at the Department of Medical Physics, Isfahan University of Medical Sciences, Isfahan, Iran, which its details and setup were described previously.[3] In brief, it consists of a gigahertz transverse electromagnetic cell which connected to GSM 900 MHz by subscriber identity module card. The RF-EMF simulating 900 MHz was performed using a pulse modulated by a 217 Hz square wave, with a 50% duty cycle. Signal generator (Model MFG-8215A) was connected through a coaxial cable to a horn antenna (transmitting, 2.4-gauge Omni-Directional Antenna), and signals were checked by an oscilloscope (Model 8203). Power density of mobile phone emission at 900 MHz was measured with an ElectroSmog Meter, TES-92. The highest SAR for human head based on the manufacturer is 0.795 W/kg. The average of SAR was 2 W/kg due to its safety limit for mobile phone radiation which was used in the previous experiments.[7] The GSM 900 MHz exposure intensity at 20 cm distance from mobile phone antenna was found to be 354.60 ± 0.03 μW/cm^2. The temperature during experiments was measured with a mercury thermometer and its variation was about 0.8°C.

Cells and exposure protocol

MCF-7 cells were purchased from cell bank of Iran Pasteur Institute, Tehran, Iran. HADSCs were isolated from human adipose tissue. Then, the characterization of HADSCs has been assessed by flow cytometry according to previous study,[9] and these cells have been frizzed in Liquid Nitrogen Storage Tanks (−1930°C).

All cells were cultured in Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum and 100 μg/ml penicillin-streptomycin in flask T75 at a density of 10^4 cell/well, and also, they were incubated at 37°C, 5% CO_2, and 95% humidity. Cultures were maintained at subconfluent levels in a 37°C incubator with 5% CO_2, and the medium was replaced every 3 days. They were passaged with trypsin/ethylenedinitrilotetraacetic acid (EDTA) (0.05% trypsin/0.53 mm EDTA) (Sigma-Aldrich) solution while cells reached 80% confluence and then were counted with a hemocytometer apparatus. Irradiated cells contain four groups. Group 1 was irradiated for 6 min/day, the second group was initially exposed for 6 min and then stopped for 10 min and irradiated for 21 min/day, the third group was under irradiation at 6, 21, and 51 min/day, and the fourth group was under irradiation at 6, 21, 51, and 101 min/day. The time interval between each exposure was defined 10 min. Control group was conducted in the same RF-EMF system without RF transmission.

3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

After exposure, the medium of each well plate was removed, washed twice with phosphate-buffered saline (PBS), and replaced with 400 μl of serum free medium and 40 μl 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solutions (5 mg/ml in PBS). Then, it was incubated for 4 h at 37°C in a 5% CO_2 incubator. The medium was removed and 400 μl of dimethyl sulfoxide (Sigma-Aldrich) was added to each well and mixed thoroughly using the pipette and incubated in a dark room for 2 h. Finally, 100 μl of medium was transferred to a 96-well plate, and absorbance of each well was read at wavelength of 570 nm with enzyme-linked immunosorbent assay reader. The results were presented as percent of cell viability.

Trypan blue test

Cell viability was performed using trypan blue test postexposure. Cells were released with trypsin EDTA, washed, centrifuged, and resuspended in a test tube. Then, a solution of trypan blue (0.4%) in PBS was prepared. Finally, cell suspension (10 μl) was mixed with of trypan blue stock solution (10 μl) in a hemocytometer and tested immediately under a microscope. In this study, the number of blue staining and the number of total cells were counted.

Statistical analysis

Three-way ANOVA test was used for parametric data statistical analysis. *Post hoc* (Tukey) test was used for the comparison of exposed and control groups. All data analyses were performed with SPSS (SPSS version 21, IBM Corp., Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant.

Results

Morphology of cells

Cell morphology study was performed with images which obtained by phase-contrast microscopy. After two or three passages with the confluency of 80%, MCF-7 and HADSCs cells appeared with their multi-dimensional and spindle-shaped fibroblastic morphology, respectively [Figures 1 and 2]. As can be seen from these figures, in both studied cells, there was no differences between morphology of controls and RF-EMF exposed groups.

Cell viability

To investigate whether RF-EMF affects the proliferation rate of isolated HADSCs and MCF-7 cells, MTT assay was performed. In MCF-7 cells, in comparison with the control group (100%), exposure to 6, 21, 51, and 101 min/day resulted in (85%, 77%, 70%, and 61%, respectively) a significant decrease of cell proliferation after 5 days ($P < 0.05$) is shown in Figure 3. Increased period (from 3 to 5 days) had a significant effect on cell viability and was less ($P < 0.01$), but the effect of changing the distance of radiation (from 10 to 20 cm) on cells survival rate was not significant ($P = 0.35$).

In HADSCs, there was no significant difference mean% of viable cells in 6 min exposed group (98.8%) as compared with the control group (100%). As indicated in Figure 4, in comparison with the control group, exposure to 21, 51, and 101 min/day resulted (69.9%, 47% and 22.4%, respectively) in a significant decrease of cell proliferation after 5 days ($P < 0.05$). Increased period of radiation time (from 3 to 5 days) had a significant effect on cell viability and was less ($P < 0.05$), but the effect of changing the distance of radiation (from 10 to 20 cm) on cells survival rate was not significant ($P = 0.09$).

Trypan blue staining

Table 1 shows that the percentage of HADSCs' cell survival in all groups was significantly lower than the control groups except in Group 6 min exposure ($P < 0.001$). Increased period of radiation exposure time (from 3 to 5 days) resulted in a significant reduction effect on cell

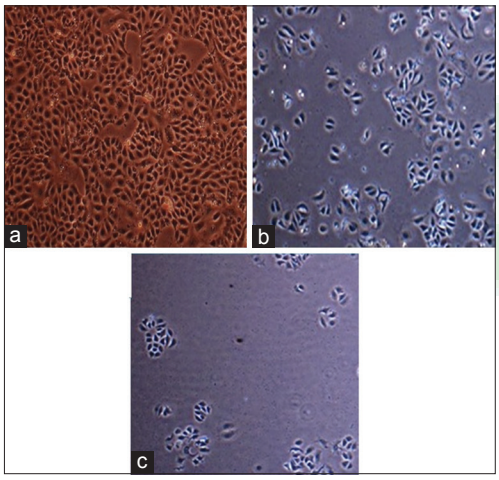

Figure 1: Cell morphology of Michigan Cancer Foundation-7 cells. (a) Control group without exposure. (b) Group 1 with 6 min exposure to radiofrequency electromagnetic field for 5 days. (c) Group 4 with 101 min exposure to radiofrequency electromagnetic field for 5 days

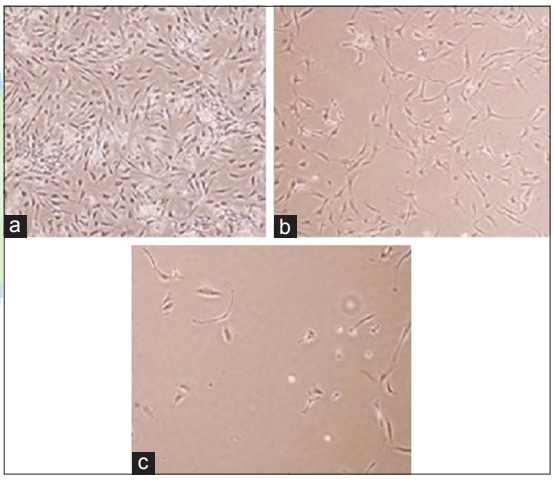

Figure 2: Cell morphology of human adipose-derived stem cells. (a) Control group without exposure. (b) Group 1 with 6 min exposure to radiofrequency electromagnetic field for 5 days. (c) Group 4 with 101 min exposure to radiofrequency electromagnetic field for 5 days

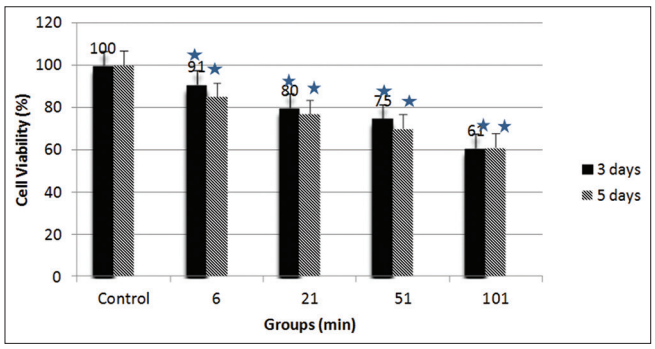

Figure 3: The percentage viability of Michigan Cancer Foundation-7 cells (mean ± standard deviation) of groups exposed to global system for mobile communication 900 MHz for different daily exposure time by 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide assay

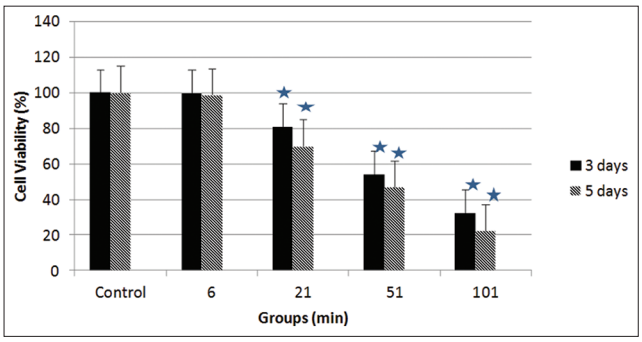

Figure 4: The percentage viability of human adipose-derived stem cells (mean ± standard deviation) of groups exposed to global system for mobile communication 900 MHz for different daily exposure time by 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide assay

viability ($P < 0.001$). There was no found any significant relationship between cell survival rate with increase of distance from radiation source (10 to 20 cm) ($P = 0.88$).

As Table 2 illustrates, in MCF-7 cells, the percentage of cell survival in all groups was significantly less than the control groups ($P = 0.03$). Increased period of exposure time (from 3 to 5 days) showed a significant effect on cell viability ($P = 0.04$). There was no found any significant relationship between cell survival rate with change of distance from radiation source (10 to 20 cm) ($P = 0.88$).

Discussion

Electromagnetic waves are nonionizing radiation due to lack of enough energy to ionize molecules in which the radiation dose will change by varying the field intensity, exposure time, the number of radiation, and radiation frequency.[8] This study evaluated the effect of irradiation time, distance, and period of radiation exposure on cell viability. MTT and trypan blue staining were used as two statistics analysis.

In this study, irradiated cells contain four groups (for both studied cells separately). As mentioned earlier (in method section), exposure times were 6, 21, 51, and 101 min/day. The time interval between each exposure was selected 10 min, and control group was conducted in the same RF-EMF system without RF transmission. All irradiation was performed with 10 and 20 cm from transmission antenna.

In MCF-7 cells, in both of MTT assay and trypan blue staining, proliferation rate and cell viability in all of the irradiation groups was significantly lower than the control groups. As identified in Table 2 and Figure 3, in exposure time longer than 21 min, reduction of proliferation rate was not significant, and this is due to the high reproductive ability of these cells. Findings here had a good agreement with results reported by Panagopoulos and Margaritis.[17]

In HADSCs, in both MTT assay and trypan blue staining, proliferation rate in all of the exposure groups except Group 6 min was significantly less than the control groups. Proliferation rate in Group 51 min exposure significantly lower than Group 21 min exposure, in addition proliferation rate in Group 101 min exposure significantly lower than Group 51 min exposure. Proliferation rate among irradiation groups decreased with increase exposure time and the frequency of the radiation. Indeed, there is also an approximately linear downward trend between the time of irradiation and the proliferation rate as indicated in Table 1 and Figure 4. The results are similar to the author previous work results using extremely low-frequency magnetic fields on proliferation rate of HADSCs.[18]

Tukey *post hoc* analysis (pairwise comparison between Group 21 and 51 min/day exposure and between Group 51 and 101 min/day exposure) showed that longer exposure time (51 and 101) reduced proliferation rate of apparent diffusion coefficients, but this difference statistically was not significant due to the proliferation of MCF-7 cells.

In this work, two different distances (10 and 20 cm) was selected to irradiation MCF-7 and HADSCs cells. From point of statistical view, the effect of distance from the antenna on the percentage of cell survival was not observed.

One of the main reasons for disparity of findings in the present work is resulted in using different cell lines with different criteria. As literature indicated, different cells have dissimilar sensitivity, cell cycle time, and response to extrinsic factors.[19-23] Hence, stem cells as undifferentiated cells with high renewable capacity were good choice for this purpose. In addition, MCF-7 cells due to theirs good reproductive properties were another choice to comparison of the results.

Furthermore, cellular responses after exposure to RF-EMF were significantly associated to cell lines rather than to primary cells. As reported before, no other experimental parameter was significantly associated with cellular responses.[18,21-23]

In vitro experiments which aim to study cellular response(s) of RF-EMF exposure need specific experimental controls to reduce confounding variables. These variables could otherwise affect the results and impair reliability and reproducibility of the experiments.[21]

Finally, comparison between the results here with the other studies revealed that there is a good agreement between

Table 1: Mean±standard deviation of human adipose-derived stem cells

Cells	Cell viability (mean±SD)	
	3 days	5 days
Control group	94.0±4.7	89.6±5.3
6 min exposure	93.0±5.5	87.0±10.7
21 min exposure	72.5±8.2	44.2±1.1
51 min exposure	63.2±3.5	32.8±6.5
101 min exposure	55.7±1.1	23.5±7.7

The percentage of cell survival in all groups except Group 6 min exposure significantly lower than the control groups ($P<0.01$). SD=Standard deviation

Table 2: Mean±standard deviation of Michigan Cancer Foundation-7 cells

Cells	Cell viability (mean±SD)	
	3 days	5 days
Control group	92.7±6.0	92.0±7.6
6 min exposure	83.8±8.5	87.2±19.4
21 min exposure	79.8±11.3	81.8±13.0
51 min exposure	73.9±13.3	74.0±13.4
101 min exposure	63.3±28.2	58.0±13.6

The percentage of cell survival in all groups significantly lower than the control groups ($P=0.03$). SD=Standard deviation

increases the risk of danger and the time of exposure even though possible hazardous effects of RF-EMF at low exposure levels are controversially discussed due to inconsistent study findings.[19,20]

Conclusions

It is concluded that GSM 900 MHz cellular phone with intensity of 354.6 μW/cm^2 at 10 and 20 cm distance from antennae for 5 consecutive days may be reduced the proliferation rate and viability of human ADSCs and MCF-7 cells regarding to the duration of exposure time, but no mechanism has been proposed to explain the effects of this radiation. It is recommended to reduce exposure time, increase distance from antenna, and reserve the use of cell phones for shorter conversations to prevent its biological and harmful effects. Of course, further investigations for assessing RF-EMF with other intensities and frequencies on different cells are suggested.

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Conflicts of interest

There are no conflicts of interest.

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